CERTIFICATE OF MAILING

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231.

Melissa Hardy (Type or print name) Melissa Hardy (Signature) July 19, 2001 (Date)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of
Heifetz *et al.*
Appl. No. 09/309,038
Filed: May 10, 1999
For: Regulation of Gene Expression

Art Unit: 1638
Examiner: A. Mehta
Atty Docket: A-30496B

DECLARATION OF JAN GIELEN UNDER 37 CFR §1.132

Commissioner for Patents
Washington, D.C. 20231

Sir:

I, JAN GIELEN, declare as follows:

1. I am a Staff Scientist with Syngenta Seeds (formerly Novartis Seeds). My credentials are set out in my Curriculum Vitae, a copy of which is attached to this Declaration as Exhibit A. I am also a co-inventor of the subject matter described and claimed in the above application.

2. On the basis of my qualifications set forth in Exhibit A, I submit that I am qualified to speak of the skill and knowledge of those skilled in the arts pertaining to the subject matter claimed in the above-identified application, particularly with respect to methods for conferring resistance or tolerance to a virus upon a cell by introducing into a cell and plant a sense and antisense RNA fragment of a viral genome and DNA constructs as described and claimed in the above-identified application.

3. I am familiar with the above-identified application and the issues raised in the Office Action dated January 19, 2001, and I make this Declaration to address the issue that the specification of the above-identified application enables the methods and DNA constructs as claimed.

4. It is my opinion that those skilled in the arts pertaining to the subject matter claimed in the above-identified application, given the description and teachings in the above-identified application and the knowledge in the art, would be able to prepare DNA constructs comprising a first and second DNA sequence capable of expressing in a cell a sense and antisense RNA fragment of a viral genome or portion thereof and further, wherein the DNA sequences are operably linked to one or more promoters. Also, one skilled in the art would be able to follow the methods described and taught in the instant specification to introduce into a cell sense and antisense RNA fragments of a viral genome or portion thereof, wherein the expression of the viral genome or portion thereof in the cell is reduced. The results of experiments conducted by myself, Elisabeth Wremerth-Weich, Britt-Louise Lennefors and Jan Bensefelt form the basis for this opinion. These experiments are described below.

5. DNA constructs were made as described in Example 9 in the present specification on pages 42-43. A schematic drawing of the resulting DNA constructs (pHiNK187 and pHiNK188) is attached as Exhibit B.

6. The DNA construct pHiNK188 was used to transform sugar beet by means of *Agrobacterium tumefaciens* according to routine procedures well-known to those skilled in the art, and as described in the specification on pages 23-24. Nuclear transformed plants resulted from the transformation. The events are listed in Exhibit C. All events carry pHiNK188.

7. The transformed plants were multiplied *in vitro*, transferred to the greenhouse and challenged with the beet necrotic yellow vein virus (BNYVV) to see if the transformed plants were resistant or tolerant to the virus. Upon acclimatization to the greenhouse conditions, sugar beet plantlets were potted in soil contaminated with *Polymyxa betae* carrying *rhizomania* (pot size: 12 cm, approximate greenhouse conditions: +22° C, 16h light). The soil was collected from rhizomania infected fields in Bayern, Germany. Four weeks after transplantation into the infected soil, the plants were pulled up and the bottom half of the roots harvested and cleaned. Using a Pollähne press, sap from 0.5 gram of root sample was collected and diluted in 10 ml extraction buffer. The virus content of the root extracts was determined using the TAS-ELISA assay from

Adgen, Scotland following the supplier's instructions. The absolute amount of BNYVV expressed in ng of virus per milliliter was calculated using a calibration standard included on each plate. The results of these viral challenge events are summarized in Exhibits C1 and C2 and Exhibit D attached hereto.

8. As shown in Exhibit C, events 279-15-A, 284-22U and 284-22-1F showed no decrease in virus content compared to the susceptible controls A and B that represent typical sugar beet lines susceptible to rhizomania, routinely used in sugar beet breeding. Events 284-22-A, -M, and -Q, however, showed a dramatic decrease in virus content and thus are highly resistant to *rhizomania* at levels superior to the resistant control C28 that consists of a sugar beet accession naturally tolerant to *rhizomania*. Finally, events 284-22-G and -I did not accumalate any virus to detectable levels and thus are considered immune to *rhizomania* infection.

Resistant plants were crossed with a pollinator heterozygous for the Holly locus, a natural source of resistance to BNYVV. The resulting progeny populations are expected to segregate for both the transgenic and the Holly locus to yield four genotypic classes: +H/–T carrying the Holly locus only, –H/+T carrying the transgenic locus only, +H/+T carrying both and –H/–T carrying neither of both loci. PCR analysis of the progeny plants for the presence of both loci allowed their classification into the four genotypes before their entry into the *rhizomania* assay. As shown in Exhibit D, all transgenic progeny plants derived from three independent events showed extreme resistance levels. Contrary to the plants homo- or heterozygous for the Holly locus, the transgenic plants hardly accumulated any virus to detectable levels, illustrating their superior levels of resistance. Only the combination of both natural sources of tolerance, the Holly and C48 locus, delivered approximately equal levels of resistance.

9. The above tests demonstrate clearly that the DNA constructs as described in the specification and claimed in the above-identified application when introduced into a cell or plant using the methods as described and claimed are capable of altering or reducing the expression of a viral genome or portion thereof in the cell or plant as described and claimed in the above-identified application.

10. Thus, in my opinion, the aforementioned experimental results demonstrate that the methods, cells, plants and DNA constructs of the claimed invention are described in such a way as to enable those skilled in this art to make and/or use the invention.

11. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true. Further, these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Subscribed to on the following date:

July 19th, 2001

JAN GIELEN